ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.31

Volume 124, pp. 31–37 *April–June 2013*

Phialophora avicenniae sp. nov., a new endophytic fungus in *Avicennia marina* in China

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Abstract — Three isolates of an endophytic fungus were obtained from *Avicennia marina*. Morphological and molecular evidence indicates that they are identical and represent a new species of *Phialophora*, described here as *P. avicenniae*. The new species is characterized by brown colonies, branched conidiophores, and catenulate conidia.

Key words — *Herpotrichiellaceae*, ITS sequence, taxonomy

Introduction

The morphologically vaguely delimited hyphomycete genus *Phialophora* Medlar is characterized by dark and slow-growing colonies, pigmented hyphae, solitary or aggregated conidiophores, and flask-shaped phialides usually with a flaring collarette. The type of *Phialophora* is *P. verrucosa* Medlar, diagnosed by its slow-growing dark olivaceous colonies and usually pigmented phialides bearing an even darker collarette (Medlar 1915). *Phialophora* has been regarded as highly polyphyletic, comprising anamorphs of discomycetes, pyrenomycetes, and loculoascomycetes (Gams 2000). Gams & Holubová-Jechová (1976) distinguished sect. *Catenulatae* with catenate conidia having a truncate base. Some members of this section and other phialophora-like species with catenulate conidia, such as *Brachyalara straminea* Réblová & W. Gams, *Exochalara longissima* (Grove) W. Gams & Hol.-Jech., and *Infundichalara microchona* (W. Gams) Réblová & W. Gams, have been segregated from *Phialophora* by molecular phylogeny analyses and morphological characters (Bogale et al. 2010; Réblová et al. 2011). *Phialophora* s. str. is recognized as a member of the *Herpotrichiellaceae* (Untereiner et al. 1995).

We obtained three isolates of an endophytic fungus from twigs of *Avicennia marina* (Forssk.) Vierh. (*Acanthaceae*) in Zhanjiang, Guangdong Province, China, in 2007. The taxonomic status of these isolates was determined using morphological and molecular methods, showing them to represent a new *Phialophora* species. The isolates are stored in the laboratory of the Agricultural College, Guangdong Ocean University, and subcultures at CBS in Utrecht, the Netherlands.

Materials & methods

Isolates

Healthy twigs (0.4–0.8 cm diam.) of *Avicennia marina* were thoroughly washed in running tap water and then sterilized by washing in 75% ethanol for 2 min and 0.1% mercuric chloride (v/v) for 2 min. The twigs were rinsed 3 times in sterile distilled water and then cut into pieces (0.5 cm in length). The small pieces were evenly spaced in Petri dishes (9 cm diam) containing a medium (Arnold et al. 2000) consisting of (per litre distilled water) dextrose 20 g, peptone 10 g and agar 20 g, with addition of 60 mg streptomycin before pouring the plates. The Petri dishes were incubated at 27°C. Hyphal tips were transferred to potato dextrose agar (PDA) dishes.

Morphological examination

Three cultures of every isolate grown on PDA plates were investigated for colony characteristics. Thirty conidia were measured under an Olympus-BX51 microscope. Scanning electron microscopy (SEM) was used to observe phialide morphology. Specimens were flash-frozen (–196°C) in liquid nitrogen under vacuum for cryo-SEM, transferred to the preparation chamber, and then to the SEM chamber where ice particles were sublimated (–80°C). Samples were sputter-coated with E-1010 in the preparation chamber for 75 sec under 2.0 KV at –170°C. Specimens were viewed under 5 KV at –188°C with a Philips SL30 scanning electron microscope.

DNA extraction, PCR amplification, DNA sequencing, and phylogenetic analyses

After the isolates were grown and incubated on PDA plates at 27°C for 10 days, the mycelia were scraped off. Genomic DNA was extracted from the mycelia using SDS procedure (Lee & Taylor 1990) with a few modifications. The quality and quantity of DNA were visually assessed by staining with ethidium bromide in 1% agarose gel electrophoresis. The partial ITS sequences were amplified according to Geiser et al. (2005) using primers ITS1 and ITS4 (White et al. 1990) in TP600 (TaKaRa PCR Thermal Cycler Dice). Shanghai Invitrogen Biotechnology Co., Ltd. sequenced the DNA. The ITS sequences of the isolates were aligned using MEGA version 4.1 (Tamura et al. 2007) and manually optimized to ensure positional homology. Gaps were considered as missing data. Seventeen sequences of *Phialophora* and one sequence of an unidentified ascomycotan species were downloaded from GenBank, and *Capronia semiimmersa* (*Herpotrichiellaceae*) was chosen as outgroup. A phylogenetic tree was constructed using the neighbor-joining method of MEGA version 4.1 (bootstrapped 1000 replicates).

Taxonomy

Phialophora avicenniae Yue L. Liu & Z.D. Jiang, **sp. nov**. Figs. 1–2

MycoBank MB561173

Differs from *Phialophora clavispora* and *P. olivacea* by its branching conidiophores and endophytic habit and from *P. oxyspora* by its ovoid to globose conidia and endophytic habit.

Type: China, Guangdong Province, Zhanjiang, endophytic in twig of *Avicennia marina*, 18 April 2007, Liu Yuelian Q37 (**Holotype**, CBS 130286 [lyophilized culture]; GenBank, GQ500118).

Etymology: named after the host genus, *Avicennia*.

Colonies initially yeast-like, gradually becoming cottony, brown, reaching 2–3 cm diam. after 5 days at 27°C on PDA plates; reaching 9 cm diam. after 14

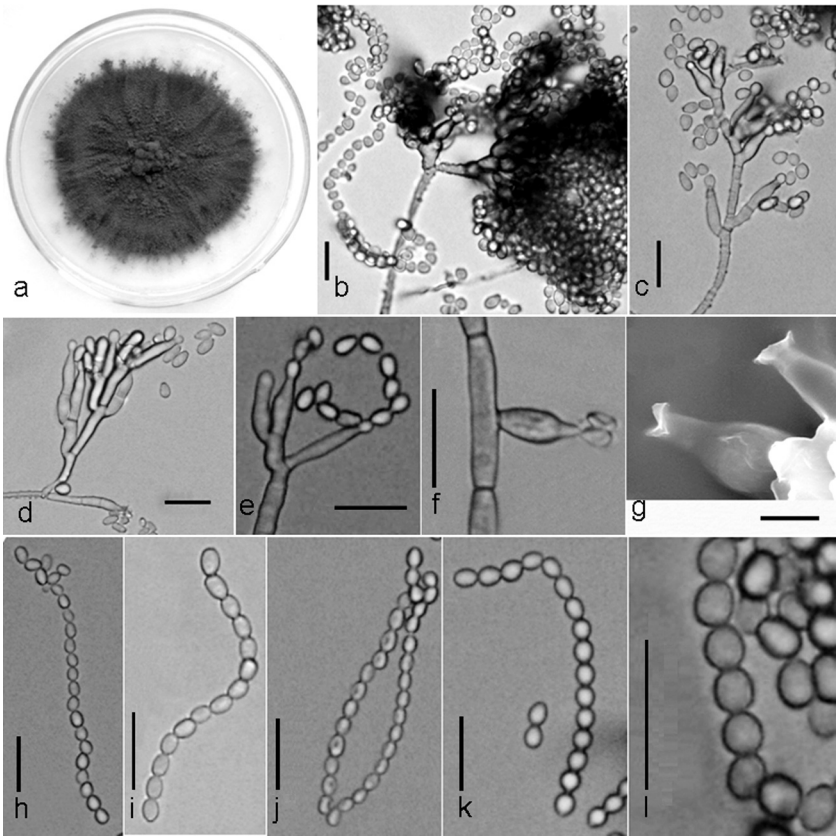

Figure 1. *Phialophora avicenniae* (CBS 1302876). a. Colony; b–f. Conidiophores; g. Phialides; h–l. Conidial chains. Scale bars: b–f, h–l = 10 μm; g = 2 μm.

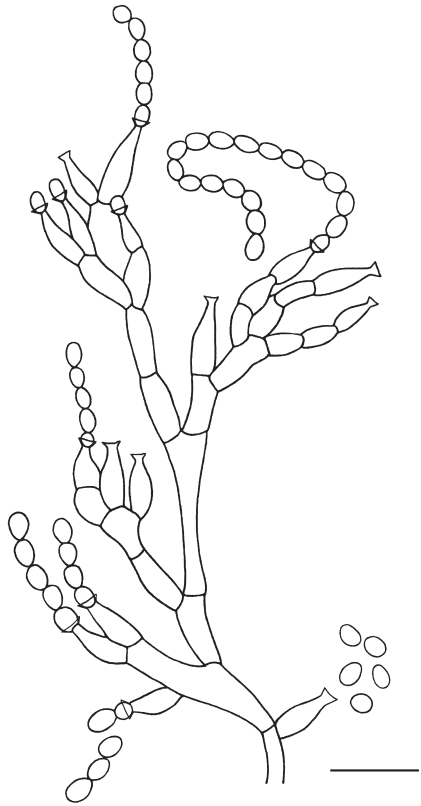

Figure 2. *Phialophora avicenniae.* Conidiophore and conidia. Bar = 10 μm.

days and changing from brown to black-brown. Hyphae hyaline to subhyaline, smooth; occasionally brown, verrucose, 1.5–3.0 μm wide. Conidiophores hyaline or brown, branched, rarely simple, septate, smooth, and variable in size. Phialides usually aggregated on conidiophores, flask-shaped or cylindrical, hyaline or brown, smooth and thick-walled, 6.5–11 × 1.8–3.5 μm. Collarette somewhat darker than the phialide body, funnel-shaped or flaring, 0.5–1.0 × 0.3–0.5 μm. Conidia catenulate, hyaline or brown, 1-celled, smooth, ovoid to globose, slightly apiculate at the base, 1.6–2.5 × 1.8–3.5 μm. Chlamydospores absent.

Teleomorph: unknown

Habitat: endophytic in twigs of *Avicennia marina*.

Additional cultures examined: **China**. **Guangdong Province**, Zhanjiang, endophytic in twigs of *Avicennia marina*, 10 August 2007, Liu Yuelian Q44 (CBS 130287; GenBank, HM055753); Liu Yuelian Q48 (CBS 130288; GenBank, HM055754).

Notes: *Phialophora verrucosa*, the type of *Phialophora*, differs from the new species by olivaceous-black colonies and broadly ellipsoidal conidia adhering in slimy heads. The new species resembles *P. clavispora* W. Gams, *P. olivacea* W. Gams, and *P. oxyspora* W. Gams by its catenulate conidia. However, *P. clavispora* and *P. olivacea* have simple conidiophores, and *P. oxyspora* has fusiform conidia (Gams & Holubová-Jechová 1976). In addition, the new species is endophytic, while the other three species are so far only known as saprobes.

Phylogenetic analyses

ITS rDNA sequences obtained from the isolates of CBS 130286, CBS 130287, and CBS 130288 were submitted to GenBank and received the

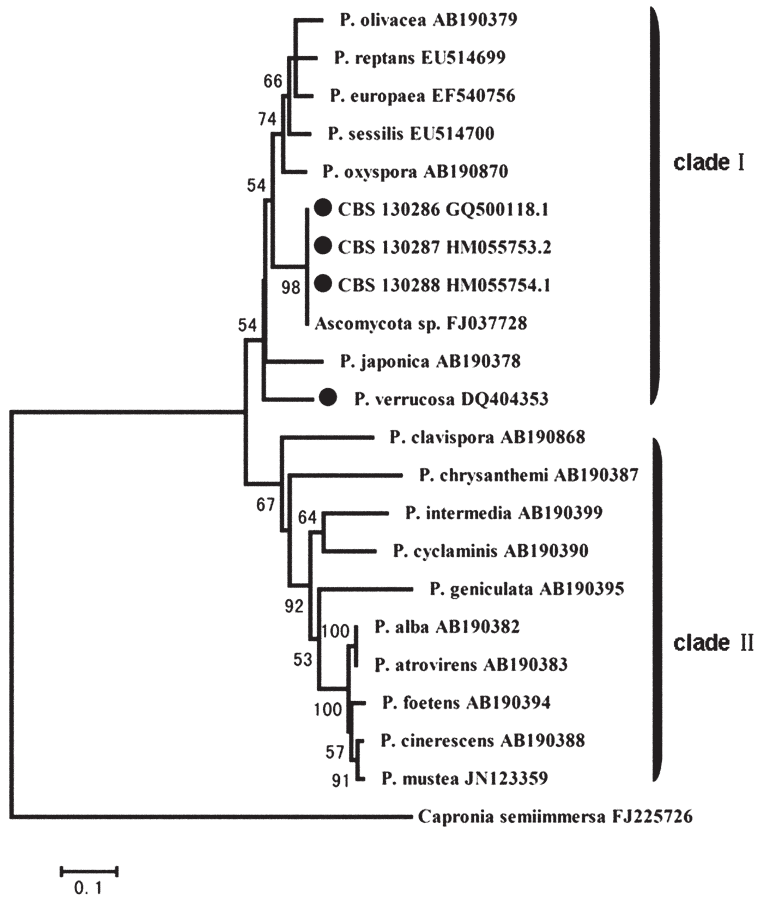

Figure 3. Phylogenetic tree based on neighbor-joining of ITS sequences, showing the relationships among *Phialophora avicenniae* and related species.

accession numbers GQ500118, HM055753, and HM055754. Blast (Basic Local Alignment Search Tool) analysis showed that the ITS sequences are 99% similar to an unidentified strain labeled "Ascomycota sp. GX6-1C" (GenBank FJ037728; isolated as an endophyte from an unknown mangrove host in China) and 79–87% similar to various GenBank isolates of *Phialophora* spp. A neighbor-joining analysis shows the relationships of the three strains with 17 other species of *Phialophora* (Fig. 3). The phylogram also shows that the unidentified strain forms a monophyletic clade (bootstrap 98%) with the three *P. avicenniae* strains, and is probably conspecific.

The *Phialophora* species shown here form two distinct clades (clade I and clade II) where *P. avicenniae* is part of clade I (bootstrap 54%), showing a sister relationship with *P. olivacea*, *P. reptans*, *P. sessilis*, *P. europaea*, and *P. oxyspora*.

Acknowledgements

We express our sincere thanks to Prof. W. Gams who revised the manuscript and deposited the strains in CBS. We also thank Prof. Yi-Jian Yao (Key Laboratory of Systematic Mycology and Lichenology, Institute of Microbiology, Chinese Academy of Sciences) for reviewing the manuscript. The authors are grateful to Dr. Martina Réblová (Department of Taxonomy, Institute of Botany, Academy of Sciences, Průhonice, Czech Republic) for providing valuable suggestions. This study was supported by the National Science Foundation of China (No. 30870015).

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